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Microarray Detection of Mosaicism in Prenatal and POC Specimens: More Complexity with Better Technology

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I. Introduction

Detection and characterization of chromosome mosaicism prenatally has always been problematic. This mosaicism usually primarily involves whole chromosome gain, although a loss of a sex chromosome may also be found. Reporting information has always been available for laboratories when detected by cytogenetic analysis. The utilization of prenatal microarray analysis has altered the standard algorithms applied in standard cytogenetic analysis due to the variety of anomalies detected and the increased frequency of detection. Microarray analysis not only detects entire chromosome mosaicism, but also elucidates other segmental CNV mosaicism as well as copy-neutral mosaicism. There has been little guidance for the latter two as to best practices when delineating mosaicism. Microarray detection of mosaicism is predicated on multiple factors: specimen type (CVS or amniotic fluid), sample type (direct or cultured), chromosome or interval size involved.

II. Methods

ARRAY METHODOLOGY: All studies were done utilizing the Affymetrix® Cytoscan® HD array [Affymetrix® and CytoScan® are Registered Trademarks of ThermoFisher Scientific.] This array contains approximately 2.695 million markers across the entire human genome. There are approximately 743,000 SNPs and 1,953,000 structural non-polymorphic probes (NPCNs). On the average there is approximately 0.88 kb between each marker. DNA was extracted utilizing standard methods and 250 ng of total genomic DNA was digested with NspI, ligated to adaptors, and amplified using Titanium Taq with a GeneAmp PCR System 9700. PCR products were purified using AMPure beads and quantified using NanoDrop 8000. Purified DNA was fragmented and biotin labeled and hybridized to the Affymetrix Cytoscan® HD GeneChip. Data was analyzed using Chromosome Analysis Suite. The analysis is based on the GRCh37/hg19 assembly.

III. Results

In general terms, mosaicism, indicates the presence of two or more populations of cells in one individual. In this study it includes any sample in which two different populations have been detected, which includes both samples with admixture as well as mosaic samples with a second genotype. The analysis of over 600 mosaic and MCC cases from ~45,000 prenatal specimens has revealed that:

- The overall frequency of MCC and mosaicism detect by microarray analysis is 1.36%
  - MCC was detected in 0.20% of the samples
  - Mosaicism was seen in 1.16% of the samples
- Admixture/MCC was detected ~twice as frequently in CVS compared to amniotic fluid specimens (0.34% vs. 0.20%)
- Mosaicism was detected ~twice as frequently in CVS compared to amniotic fluid specimens (1.89% vs. 0.92%)
- Five major types of mosaicism was detected in these studies (aneuploidy, marker chromosomes, structural abnormalities; segmental UPD and whole genome mosaicism – **Figure 1**)
  - Aneuploidy was the most common, seen in ~50% of the mosaic cases
- 26.2% of the cases in which mosaicism was detected by the array could not be confirmed
  - Confirmation was slightly higher in AF samples than CVS samples
  - Approximately 33.3% of the mosaicism detected in both AF cultures and CVS direct specimens could not be confirmed, whereas only 15.1% of the AF direct specimens mosaics could not be confirmed
- Of those cases that confirmed, an extended analysis needed to be done in ~15.5% of the cases to achieve confirmation
- Approximately 8.7% of the confirmed aneuploidy also showed Uniparental disomy, indicating that the mosaicism was due to a rescue event (**Figure 2**)
- 77 chromosomally abnormal mosaic CVS cases were studied by microarray analysis on amniotic fluid; only 3.9% could be confirmed

Figure 1: Distribution of Types of Mosaicism Detected Prenatally

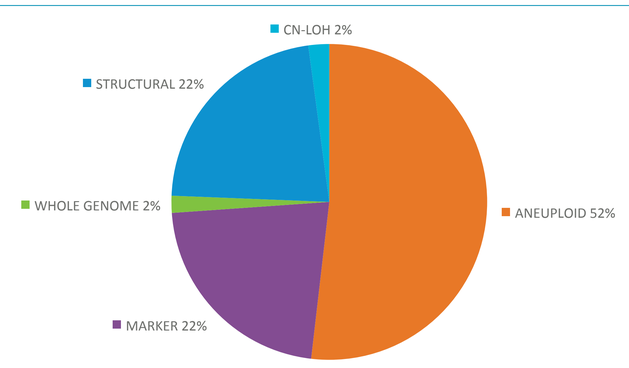
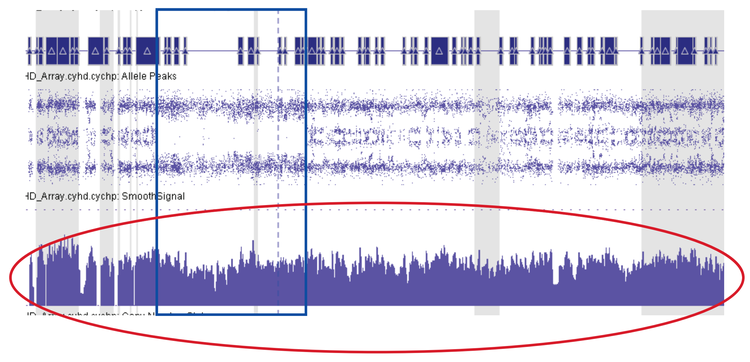


Figure 2: Mosaicism and UPD of Chromosome 15



IV. Discussion

Amniocentesis – have detected low level mosaicism that may not have been seen by chromosome. Microarray detection of mosaicism must be predicated on three factors:

- Specimen type - Amniocentesis vs. CVS
- Sample type - Direct specimen vs. culture
- Chromosome involved

Regardless of specimen and sample type – microarray analysis can only be considered as a result coming from one "culture". CVS – results mimic a combination of direct and cultures cells and the type of CPM cannot be determined. Samples from direct preparations include both cytotrophoblasts and mesenchyme.

- All of these cases, whether confirmed by cytogenetics, should be confirmed by amniocentesis
- If from a CVS culture; it could be true, pseudomosaic or cultural artifact and should be confirmed by amniocentesis

If it is an amniotic fluid sample, it is viewed differently depending if a direct or a culture. If a culture, it may be true mosaicism or pseudomosaic or possible a cultural artifact.

- Both FISH and cytogenetic results should be reviewed
  - Report if confirms
  - Don't report if not confirmed if low percent
    - This should be re-evaluated if higher percentage of trisomy 21, 13 or 18

If from a direct preparation

- Report if confirms
- Report but cautiously if it doesn't confirm - May be placental in origin

V. Conclusions

These studies have yielded interesting findings and have assisted in providing directions on reporting of these unusual and intriguing results including:

1. The detection of whole chromosome gain/loss mosaicism is increased by microarray studies (over standard cytogenetics) and mosaicism can be detected at frequencies as low as 5%;
2. Mosaicism that has been detected by microarray analysis cannot always be confirmed by standard chromosome analysis, however when it has been confirmed, some of the time it was not initially detected by routine chromosome analysis and the analysis of additional metaphase cells was needed;
3. In some patients the mosaicism was detected only in a direct microarray analysis and not confirmed by chromosome analysis resulting in a more problematic counseling session;
4. In addition to the detection of mosaicism involving whole chromosome, mosaicism involving segmental CNVs too small to be detectable by chromosome analysis, have been identified and confirmed;
5. In our laboratory segmental mosaic UPD have been identified, many associated with Beckwith-Wiedemann syndrome;
6. Prenatal, POC and postnatal analyses have also revealed mosaic terminal deletions. Additional studies in a subset of these cases have demonstrated that they result in a normal copy number through post zygotic correction of these terminal deletion;
7. In some cases, initial utilization of NIPT demonstrated the underlying placental mosaicism;
8. In addition to the aforementioned mosaicism, several patients with mosaic chimeric whole genome wide UPD (androgenic and digenic chimerism) has been delineated in our patient population;
9. Both segmental CNVs and UPD in addition to whole chromosome aneuploidy have been demonstrated in samples from both direct preparations as well as from cultures, however some of the latter cases have been demonstrated to be as cultural artifacts demonstrating the superiority of direct analysis;
10. These studies have clearly demonstrated the array is more effective than standard cytogenetics in detecting mosaicism and many mosaic abnormalities would have been missed by routine cytogenetic studies.

Lastly, based on these studies, we have developed an algorithm for approaching prenatal mosaic cases detected by microarray analysis. This approach is based on the percent mosaicism, chromosome (segment) involved, whether it is from a chorionic villous sample or amniotic fluid and whether it is a direct or cultured specimen.

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